Mussel Adhesive Protein (MAP), Cell Culture Grade

Cat. # MAP-O4012

For Research and Further Cell Culture Manufacturing Use, Not For In Vivo Or Diagnostic Use.

DESCRIPTION

Mussel Adhesive Protein (MAP), Cell Culture Grade is an effective bioadhesive with wide spectrum. MAP adhesive is a formulation of the "polyphenolic proteins" extracted from the marine mussel, Mytilus edulis. This family of related proteins is the key component of the glue secreted by the mussel to anchor itself to solid structures in its natural environment. MAP adhesive will readily coat a variety of materials, such as glass, plastics and even metals. The coating is transparent and stable for 3-30 days at 2-8℃.
MAP adhesive is designed to be used as a coating on a substrate to immobilize cells or tissue. It can simplify the manipulation of biological samples in a number of common in vitro techniques, including: Establishment of primary cultures: In situ hybridization, Immunoassays, Microinjection and Immunohistochemistry.

FORMULATION

1 mg/ml in 1% acetic acid.

ENDOTOXIN

Less than 0.02 EU per μg by the LAL method.

PACKAGING

Polypropylene vial containing 1 mg.

STABILITY

Stable for a minimum of 12 months from day of production when stored at 2-8℃.

STORAGE

Store at 2-8℃

COATING METHODS

There are primarily two methods for coating a surface with MAP adhesive: hand-spreading and adsorption. In general, adsorption is recommended because it is more consistent and convenient than hand-spreading. But hand-spreading has its place for special situations.

Hand-spreading: The MAP protein can be deposited on a glass or plastic surface by mechanical spreading. Using a handy tool, such as a glass rod or micropipette tip, microliter volumes of MAP in 1% acetic acid can be spread in a thin liquid film. As the acetic acid evaporates, a coating of MAP is left behind. After washing with ethanol and water, the vessels are ready to use. Although hand-spreading gives a less uniform coating than adsorption, it may be useful in special situations, for example when only a portion of a dish or glass slide is to be coated.

Adsorption: The simplest and most cost-effective method of applying MAP is by adsorption from a neutral solution. The method is based on the observation that MAP comes out of solution as the pH is raised and spontaneously adsorbs to the first surface it contacts.
The resulting coating is quite thin (probably close to a protein monolayer) and more uniform than that achieved by hand-spreading. The major advantages of this adsorption method are: coating vessels of any shape is simple; less MAP is required per cm2 of surface area; greater flexibility - the initial concentrations and adsorption times can be adjusted to best suit.

Several Precautions Should be Taken when Using the Adsorption Method: Adsorption begins immediately upon changing the pH; therefore, after diluting the MAP into the neutral buffer, dispense within 10 minutes; pH appears to be the most important variable regulating adsorption (a final pH between 6.5 - 8.0 is optimal). Although most buffers can be used to neutralize MAP, sodium bicarbonate works best. If necessary, a volume of 1N NaOH equal to half the volume of MAP used may be added in combination with a neutral buffer to bring the solution to neutral pH; Avoid introducing neutral buffer into the MAP stock solution via a contaminated pipette or other means. Contamination of stock solution will result in precipitation of MAP onto surface of stock vial with resultant loss of product.

BASIC ADSORPTION COATING PROTOCOL

- Prepare a neutral buffer solution. 0.1 M sodium bicarbonate, pH 8.0 is recommended when coating aseptically. Filter-sterilize the buffer.
- Calculate amount of MAP required From the size and number of vessels to be coated, calculate total surface area. The best density of MAP depends on your specific application, or cell type. A preliminary dose-response experiment is recommended to determine optimal density. Otherwise, start at a density of 3.5 μg MAP/cm2 of surface area. High densities will not necessarily improve performance, so the “minimum effective density” should be determined empirically.
- Neutralize MAP and dispense to the vessel. The exact volume of buffer will depend on whether the sides of vessels will be coated. Dilute the correct amount of MAP into the buffer, mix thoroughly, and dispense within 10 minutes. Note: If the pH in the coating buffer is not between 6.5 - 8.0, MAP will not perform optimally. An aid to attaining this pH window is to use a volume of 1N NaOH equal to half the volume MAP solution used in combination with a neutral buffer.
- Incubate for adsorption. A minimum incubation of twenty minutes is recommended, but longer time will not have a negtive effect on adsorption, even if all the liquid evaporates. Pour off MAP and wash with sterile water to remove bicarbonate.

Please contact us via ***TechSupport@acrobiosystems.com***, if you have any question on this product.

The coated vessels includs the following:

- Culture dishes
- Culture flasks
- coverslips and slides
- Multiwell plates
- Cell Culture Inserts

IMMOBILIZATION OF CELLS AND TISSUE

Procedures for Seeding Cells:

Both anchorage-dependent and non- anchorage-dependent cells stick when they come in contact with MAP coated surface. In fact, the cells need not be even be viable. Fixed cells can also be immobilized, as well as microrganisms, such as yeast and bacteria.

Occasional problems with attachment have been traced to the fact that some cells (e.g., adipocytes, HL-60) and microorganisms do not readily settle out of suspension.

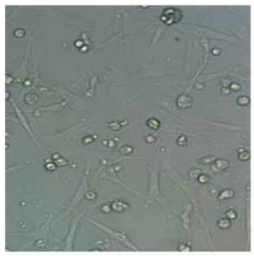
In this case, reduce the total volume of medium in which the cells are seeded until it just covers the bottom of the vessel. If this does not improve performance cells might have to be centrifuged onto the surface.

Cells may be seeded in serum-containing medium, but do not preincubate the medium in the MAP -coated vessel before adding the cells. Serum proteins can block the adhesive sites. To increase cell attachment kinetics, try seeding cells in serum-free medium. Change to serum-containing medium immediately after cells attach.

Adhering Tissue Sections:

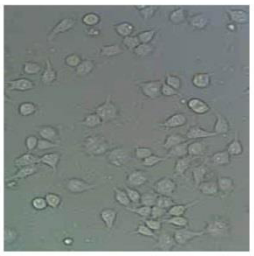
Use standard techniques to pick up cryostat or paraffin tissue sections on a MAP -coated slide. After mounting the section, remove excess moisture with a paper towel and transfer the slide to a 45℃ warm table for at least one hour. Process the slide as required by the specific protocol.

REPRESENTATIVE FIGURE FOR DIFFERENT CELLS CULTURE



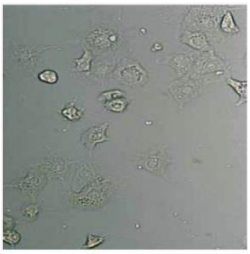
MAP (12.5μg/ cm^2)

BEK-21 at 12 hours after seeding



MAP (12.5μg/ cm^2)

HEK at 12 hours after seeding



MAP (3.5μg/ cm^2)

MSC at 2 hours after planting

REFERENCES

1. Waite, J.H. and M.L. Tanzer, 1981, Science, 212:1038-1040.
2. Waite, J.H.,1983, Journal of Biological Chemistry, 258:2911-2915.

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